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EPIDEMIOLOGY

Does Simian Virus 40 Cause Non-Hodgkin Lymphoma? A Review of the Laboratory and Epidemiological Evidence

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Recently, several studies have reported the detection of DNA from simian virus 40 (SV40), a macaque polyomavirus, in tumor tissues obtained from non-Hodgkin lymphoma (NHL) patients. SV40 accidentally contaminated poliovirus vaccines administered to millions of individuals in 1955–1962. A link between SV40 and NHL is biologically plausible because SV40 causes hematological malignancies in laboratory rodents. However, detection of SV40 DNA in human NHL tumors has not been confirmed by other laboratories. Casting doubt on an association between SV40 and NHL, follow-up studies of recipients of SV40-contaminated poliovirus vaccines have not revealed these individuals to be at increased risk of NHL. Furthermore, 2 recent case-control studies of NHL documented only infrequent SV40 antibody reactivity among NHL cases, and the prevalence of SV40 antibodies was similar in cases and controls. This review summarizes recent laboratory and epidemiological studies bearing on the question of whether SV40 is a cause of NHL in humans. The strengths and weaknesses of these data are discussed, and a framework for considering the collected evidence is presented. Many of the considerations raised in this review apply to the evaluation of data regarding other cancers, such as mesothelioma, brain tumors, and various sarcomas, for which an etiologic link with SV40 also has been proposed.

Keywords Simian virus 40; Non-Hodgkin lymphoma; Poliovirus vaccines; SV40 antibody

INTRODUCTION

Non-Hodgkin lymphoma (NHL) incidence has increased dramatically in recent decades in the United States. The cause of NHL is largely unknown, but viruses, such as human immunodeficiency virus (HIV) and Epstein–Barr virus, are etiologically important in some cases.[1] Several groups have reported the detection of DNA of simian virus 40 (SV40), a macaque polyomavirus, in tumor tissues from NHL patients.[2–7] A link between SV40 and NHL is biologically plausible because SV40 causes leukemia and lymphoma in laboratory rodents.[8] Tumors could arise through the actions of T antigen, an SV40 protein that can inactivate cellular tumor suppressor proteins p53 and pRb.[9] However, SV40 has not been shown to be capable of infecting human lymphocytes.[10] Also, additional studies of NHL tumors from humans have not confirmed the detection of SV40 DNA.[11,12]

Further complicating interpretation of these laboratory data, the origin of the SV40 DNA sequences detected in tumor tissues remains unclear. SV40 infections conceivably might have occurred via receipt of poliovirus vaccines during 1955–1962, when SV40 was a frequent contaminant of vaccines grown in monkey kidney tissue. Tens of millions of people in the United States, predominantly children, were exposed to SV40 through large-scale vaccination campaigns during years when poliovirus vaccine was contaminated with SV40. Following the discovery of SV40 and changes in vaccine production, poliovirus vaccine lots released in the U.S. from 1963 onward have been free of this virus.[13]

Given the widespread exposure to SV40 in vaccination campaigns, the question of whether SV40 causes cancer has public health importance. Nonetheless, epidemiological studies consistently have shown no relationship between exposure to this virus and cancer. Two types of epidemiological studies offer complementary approaches to the question of whether SV40 causes cancer. The first type of study, the retrospective cohort study, involves the follow-up of large groups (i.e., cohorts) of SV40-exposed and unexposed individuals for the occurrence of cancer. Retrospective cohort studies have used the receipt of SV40-contaminated poliovirus vaccine as a marker for SV40 exposure. If SV40 causes cancer, one would expect higher cancer incidence among vaccine recipients than among individuals who did not receive vaccine. The second type of study, the case-control study, evaluates individuals with and without the cancer of interest for evidence of prior SV40 exposure or infection. In these studies, SV40 status is assessed through the measurement of an antibody response to the virus.

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This review summarizes recent laboratory and epidemiological studies bearing on the question of whether SV40 is a cause of NHL in humans. The strengths and weaknesses of these data are discussed, and a framework for considering the collected evidence is presented. Although the question of whether SV40 might cause human malignancies other than NHL (e.g., mesothelioma, diverse brain tumors, various sarcomas[14]) is not a focus of this review, many of the considerations raised in this review apply to the evaluation of data regarding these other cancers.

LABORATORY STUDIES REPORTING ON DETECTION OF SV40 IN HUMAN TUMORS

Laboratories that have used sensitive polymerase chain reaction (PCR) methods to detect SV40 DNA in NHL tumor tissue have described variable results (Table 1). Four U.S. studies reported detecting SV40 T antigen sequences in tumor tissue from 15–43 percent of NHL patients.[2–5] SV40 T antigen sequences also were found in NHL tissues from 11 percent of Japanese patients.[7] Additionally, SV40 sequences were detected in 14 percent of NHL specimens from Italy.[6] On the other hand, 2 other European studies of patients with lymphoproliferative disorders were strikingly negative.[11,12] In a study based in Italy and Spain,[11] SV40 T antigen sequences were found in only 3 percent of tissues using 2 sets of PCR primers, and none of the initially positive specimens was positive using a third, confirmatory primer set. In a study from the United Kingdom,[12] SV40 T antigen sequences were not detected in any NHL specimens using quantitative real-time PCR, despite a detection limit of 10 SV40 copies in 100 ng genomic DNA (i.e., approximately one SV40 copy per 1,500 cells).

The reasons for this variability in results from PCR-based studies are unclear. Although varying results from different countries might reflect geographic differences in the patient populations, the results varied somewhat even among the U.S. studies[2–5] and the Italian studies.[6,11] Differences in PCR testing methods, such as the selection of PCR primers or reaction conditions, may have contributed to the variability in results (Table 1). Importantly, some laboratory studies suffered from methodological problems, including a lack of masked evaluation of tumor specimens,[2–7,11] lack of appropriate human tissues as negative controls,[2,5–7,11] and the amplification of SV40 sequences from negative control tissues.[3] The same problems have plagued studies reporting the detection of SV40 DNA in a wide range of other human cancers.[14] Indeed, the extreme diversity of tumor types in which SV40 DNA has reportedly been detected has complicated interpretation of these data. Similarly, studies describing the presence of SV40 DNA in NHL tumors have found sequences in NHLs of diverse histologic subtypes, including follicular, diffuse large B cell, Burkitt, and T cell NHLs.[3,4]

Contributing to difficulties in interpretation, SV40 DNA sequences are present in over 200 cloning vectors and plasmids used by laboratories worldwide,[15] which could cause contamination of tumor tissues during laboratory evaluation. Vilchez et al.[4] reported the amplification of

TABLE 1
PCR-based detection of SV40 in human non-Hodgkin lymphoma tumors

Study	Country	No. positive/ no. tested (%)	PCR primers*	PCR cycles	Tissue type†	Neg. controls/ masking‡
Martini, 1998[6]	Italy	13/95 (14)	PYVfor/PYVrev, T3/T4, RA3/RA4	Not stated	Not stated	No/No
Rizzo, 1999[2]	U.S.	5/34 (15)	SV5/SV6	45	Fresh	No/No
David, 2001[5]	U.S.	15/79 (19)	SVfor/SVrev, VPF/VPR	35	Not stated	No/No
Shivapurkar, 2002[3]	U.S.	29/68 (43)	SVTagP1/SVTagP2 (P3), SVfor2/SVrev	45	Fresh, fixed	Yes/No
Vilchez, 2002[4]	U.S.	64/154 (42)	PYVfor/PYVrev	45	Not stated	Yes/No
Capello, 2003[11]	Italy, Spain	17/500 (3)	SVTagP1/SVTagP2, SVfor2/SVrev, TA1/TA2	45	Fresh	No/No
Mackenzie, 2003[12]	U.K.	0/152 (0)	3521–3543/3577–3598, PYVfor/PYVrev	40	Fresh	Yes/Yes
Nakatsuka, 2003[7]	Japan	14/122 (11)	SVTagP1/SVTagP2 (P3)	45	Fresh	No/No

*See referenced studies for definitions of primer pairs.

†Tissue type fresh = fresh frozen; formalin = formalin-fixed paraffin-embedded; ND = not described.

‡Negative controls = inclusion of biological tissues as negative control; blinding = documentation that both extraction of DNA and PCR were done under masked conditions.

SV40 DNA from NHL tumors that was similar in sequence to that amplified from a vial of poliovirus vaccine preserved from 1955, arguing against contamination as a ready explanation. Nonetheless, the amount of SV40 DNA detected in PCR-positive NHL specimens appears to have been extremely low, as indicated by semiquantitative PCR,[6] by the inability to confirm positive results with additional primers or Southern blotting[2,5,11] and by the negative results reported by investigators using a highly sensitive PCR assay.[12] Because SV40 is integrated into chromosomal DNA in animal tumors and present within each tumor cell,[8,16] the reported detection of SV40 DNA at much lower levels in human tumors raises the possibility of PCR contamination or other laboratory artifact.[17] Indeed, Lopez-Rios et al.[18] recently provided strong evidence that at least some detections of SV40 DNA in mesothelioma tumors are attributable to plasmid contamination of PCR reactions.

Unfortunately, data from studies using other methods to detect SV40 in NHL tumor tissues also have been inconsistent. Nakatsuka et al.[7] described the localization of SV40 sequences within NHL tumor cells using in situ hybridization. On the other hand, SV40 T antigen protein was not detected by immunohistochemistry in any of 232 NHLs from France and Canada.[19] Overall, the accuracy and reproducibility of laboratory methods for detecting SV40 in human tumors remain uncertain.[14]

RETROSPECTIVE COHORT STUDIES OF POLIOVIRUS VACCINE RECIPIENTS

A recent retrospective cohort study from Denmark provides relevant data on cancer risk associated with SV40 exposure.[20] Poliovirus vaccination campaigns began in Denmark in April 1955, using a parenteral, formalin-inactivated poliovirus vaccine (IPV). Because of the urgent need to control the ongoing poliomyelitis epidemic, public health authorities conducted a series of coordinated poliovirus vaccination campaigns through the early 1960s. As of April 1962, approximately 90 percent of children aged 9 months or older had received at least one dose of IPV, and most children had received 3–4 doses.

SV40 contamination of poliovirus vaccines arose in this era because vaccine virus was cultured in kidney tissue from macaques that frequently were infected with SV40. Formalin added in the final steps of vaccine manufacture to inactivate poliovirus did not completely inactivate SV40 because SV40 is relatively resistant to this treatment.[21] Notably, Danish poliovirus vaccine was grown in monkey kidney tissue using a monolayer method that pooled together kidney cells from dozens of monkeys.[20] This manufacturing method greatly increased the likelihood of SV40 contamination of the final vaccine product. Indeed, testing in late 1961 revealed that 9 of 9 evaluated lots of Danish IPV, previously released for use in vaccination campaigns, contained live SV40.[20] Vaccine production was halted, and beginning in 1963, all Danish IPV was free of SV40.

Thus, almost all Danish children alive between 1955 and 1962 were exposed to live SV40 on multiple occasions through injection, while children born in 1963 and after were unexposed. In a retrospective cohort study,[20] Engels and colleagues examined cancer incidence in 3 Danish cohorts, defined by year of birth, who had varying exposures to SV40-contaminated IPV: the 1946–1952 birth cohort, who were vaccinated in 1955 when IPV first became available (exposed as children); the 1955–1961 birth cohort, most of whom were vaccinated at approximately 9 months of age (exposed as

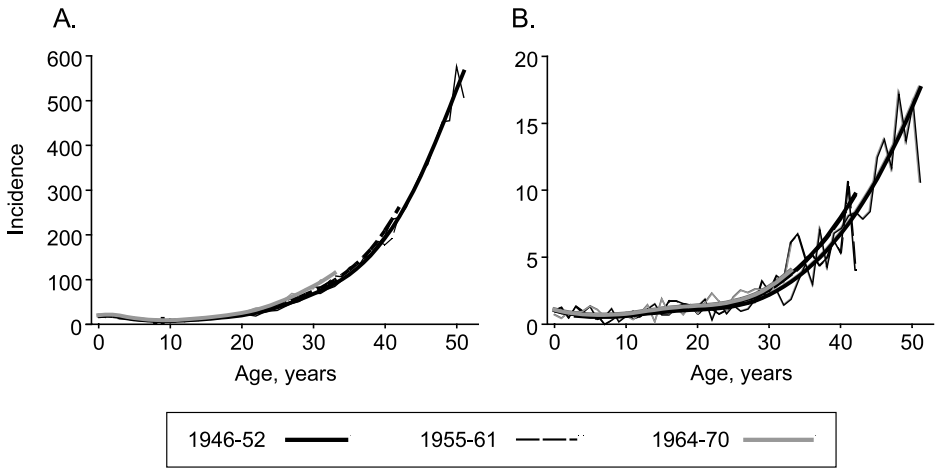

FIG. 1. Age-specific cancer incidence for 3 Danish birth cohorts with varying exposure to SV40-contaminated poliovirus vaccine. Incidence data are shown for all cancers combined (panel A) and for non-Hodgkin lymphoma (panel B). The 3 birth cohorts are 1946–1952 (exposed to SV40-contaminated poliovirus vaccine as children, solid black line); 1955–1961 (exposed to SV40-contaminated poliovirus vaccine as infants, dashed black line); and 1964–1970 (unexposed, solid gray line). Both observed incidence (thin lines) and fitted estimates derived from a Poisson model (thick lines) are shown. Incidence is per 100,000 person-years (vertical scales differ in the 2 panels). Figure is modified from Engels et al.[20] (used with permission from Oxford University Press).

infants); and the 1964–1970 birth cohort, who were born after vaccines were cleared of SV40 and, thus, were unexposed.

Figure 1 depicts the incidence of all cancers combined and of NHL for these 3 birth cohorts, using data derived from the Danish cancer registry. The 2 cohorts exposed to SV40-contaminated IPV as infants or children actually had lower overall cancer incidence than the unexposed cohort. The incidence of NHL was similar in the cohort exposed as infants and in the unexposed cohort (age-adjusted relative risk 0.93, 95%CI 0.78–1.11), while incidence was lower in the cohort exposed as children than in the unexposed cohort (age-adjusted relative risk 0.79, 95%CI 0.66–0.95). Analyses for other cancer outcomes produced similar results, that is, cancer incidence was not elevated in the 2 SV40-exposed cohorts.[20]

These null results, and the similar results of other retrospective cohort studies of vaccine recipients,[22,23] argue against an important effect of SV40 on the risk of NHL. Animal experiments demonstrate that SV40 is most oncogenic when infection occurs early in life.[24] It is, therefore, especially telling that retrospective cohort studies of individuals who received SV40 contaminated poliovirus vaccines as infants or children have not found an increased risk of cancer related to this exposure.[20,22,25,26] Of further note, most children received multiple doses of poliovirus vaccine and would have been exposed to SV40 on multiple occasions. Another feature of some studies,[20,25,27] of value for studying rare cancer outcomes arising many years after SV40 exposure, is their use of cancer registries, which has allowed the long-term follow-up of millions of vaccine-exposed individuals.

It is important to note that the retrospective cohort studies rely on several assumptions which point to some limitations of these studies.[14] First, the studies depend on the demonstration that poliovirus vaccines contained live SV40. While there are good data to support this conclusion for Denmark,[20] it is clear that contamination of U.S. IPV was more sporadic.[13] Still, the multiple doses of vaccine received by children would have increased the likelihood of SV40 exposure.[28]

Second, it is unknown how frequently exposure to SV40 present in these vaccines led to actual infections, that is, persistent presence of SV40 within the host. Limited data from Denmark indicate that receipt of Danish IPV was associated with SV40 seroconversion (3 of 3 evaluated individuals developed SV40 neutralizing antibodies following vaccination).[20] In other countries, SV40 seroconversion rates following IPV vaccination varied widely, perhaps reflecting variable amounts of live SV40 present in vaccines or differing antibody assay methods.[13] SV40 seroconversions might have reflected, at least in part, an immune response to formalin-inactivated viral proteins or an abortive SV40 infection. Still, one might expect that, if humans can be infected by SV40 by any route, exposure by injection on multiple occasions at a very young age, as experienced by many IPV recipients in the 1955–1962 period, would have led to infection.

Finally, because these cohort studies compare persons who received SV40-contaminated vaccine with those who did not, a further assumption is that SV40 infection was rarely acquired by other routes and that SV40 transmission effectively ceased in 1963 with clean-up of the poliovirus vaccines. No epidemiological study has been conducted to determine whether SV40 presently infects humans or is transmitted among asymptomatic persons. Transmission routes for polyomaviruses, such as BK and JC viruses in humans and SV40 in macaques, are not well established. BK and JC viruses are often shed in human urine and found in sewage,[29,30] whereas SV40 is not readily detected.[29,30] Approximately 2–12 percent of healthy individuals manifest SV40 antibodies, but these likely represent cross-reactive antibodies to BK or JC.[31] Together, the lack of shedding of SV40 in human urine and infrequent detection of SV40-specific antibody responses suggest that SV40 is not a common human infection.

These considerations suggest an overarching question, yet unanswered, regarding whether humans can be infected with SV40 at all. Nonetheless, to the extent that these assumptions—that is, that children in 1955–1962 widely were exposed to live SV40 and that exposures ceased in 1963—are met, retrospective cohort studies such as the Danish study provide a valid test of the hypothesis that SV40 is responsible for cancers in humans. The retrospective cohort studies make use of the unique circumstances for children in 1955–1962, for whom exposure to SV40 was highly prevalent and occurred early in life, when infection might be prone to induce cancer.

CASE-CONTROL STUDIES

Case-control studies have 2 advantages over cohort studies. First, through the recruitment of relatively large numbers of individuals with the cancer of interest, case-control studies allow an evaluation of associations for rare cancer outcomes. Second, case-control studies facilitate collection of data on SV40 exposure or infection that would be infeasible for the much larger number of individuals included in cohort studies. Along these lines, the measurement of SV40 antibodies in case-control study subjects provides a direct assessment of SV40 exposure status. Animals with SV40-induced cancers produce high levels of antibody to SV40 capsid proteins and T antigen.[7,32] Therefore, if SV40 causes cancer in humans, one would expect a high prevalence of SV40 antibodies in cases, and that this prevalence would be noticeably higher in cases than controls.

De Sanjose et al.[33] conducted a case-control study of lymphoma in Spain. Cases with NHL (N=463) or Hodgkin lymphoma (N=57) were enrolled from 4 hospitals in Spain. Controls (N=587) were individuals treated for a variety of other medical conditions at the same institutions. The authors

measured antibodies against SV40 in these individuals using an enzyme immunoassay incorporating SV40 virus-like particle (VLPs). VLPs are empty capsids, generated by spontaneous self-assembly of the major capsid protein VP1, which immunologically resemble native virions. This enzyme immunoassay is highly sensitive and specific in detecting SV40 infection in macaques.[31,34]

In the de Sanjose study,[33] SV40 seroprevalence actually was marginally lower in the lymphoma cases (5.9 percent) than in controls (9.5 percent), yielding an odds ratio of 0.61 (95%CI 0.38–0.95). SV40 seroprevalence was similarly low for NHL overall (4.3 percent) and for various NHL subtypes when these were considered separately. Furthermore, the levels of SV40 antibody present in seropositive subjects, as measured by enzyme immunoassay, were much lower than seen in SV40-infected macaques, and these levels decreased substantially when subjects' sera were incubated with BK virus VLP. These results indicate that much of the observed SV40 seroreactivity in humans likely was due to cross-reactivity with BK virus, a common infection of humans. Thus, the study provided evidence that the risk of NHL was not related to exposure to or infection with SV40. Similar conclusions were reached in a recent population-based case-control study of NHL in the U.S.[35]

Case-control studies of other cancers likewise have revealed only low SV40 seroprevalence among cases, and no difference in seroprevalence between cases and controls.[26,36–38] The low frequency of antibody to SV40 in cases (approximately 5–10 percent) contrasts strikingly with the high rates of PCR-based detection of SV40 reported in some tumor studies. Indeed, the low level of SV40 reactivity and the observed cross-reactivity of the SV40 antibodies with human polyomaviruses BK and JC suggest that very few cases or controls actually are infected with SV40.[31]

A strength of the case-control studies is that they yield a measure of association (the odds ratio) that corresponds to an estimate of cancer risk related to exposure to or infection with SV40. In contrast, studies that depend exclusively on laboratory evaluation of human tumor tissues (e.g., PCR testing of NHL tumors) cannot yield a similar measure of association because the concomitant evaluation of control tissues (e.g., PCR testing of normal lymph nodes) has not been shown to provide a reliable measure of infection prevalence in controls.

SV40 AND NHL IN HUMANS: EVALUATING THE EVIDENCE

In the preceding sections, this article reviews some of the complex and, at times, contradictory data regarding the possible relationship between SV40 and NHL. These results, from multiple lines of evidence, are not synthesized easily. In broad terms, while animal studies indicate that SV40 can cause cancer in animals and some laboratory studies have found evidence for SV40 in human tumors, epidemiological studies (cohort studies of vaccine recipients, case-control studies) have not identified an association between SV40 exposure or infection and human cancer. Overall, what do these conflicting data suggest?

In 1965, Hill[39] outlined 9 criteria for evaluating possible causal associations between disease-causing agents and human disease. The Hill criteria are a useful framework for evaluating the available evidence regarding SV40 and NHL (Table 2). As applied to the question of SV40 and NHL, these criteria and the evidence bearing on them are as follows:

1. *Biological plausibility.* It is biologically plausible that SV40 could be a cause of NHL because SV40 causes NHL in experimental animals. However, SV40 has not been shown to be capable of infecting human lymphocytes,[10] which lessens plausibility.
2. *Analogy.* SV40 might be a cause of NHL in humans because of the analogy with experimental animal models—SV40 causes NHL when injected into laboratory rodents.
3. *Biological coherence.* According to Hill, ''the cause-and-effect interpretation of our data should not seriously conflict with the generally known facts of the natural history and biology of the disease'' (page 298). There are two aspects of the hypothesis that SV40 causes NHL in humans that do not cohere with accepted biological principles. First, the amount of SV40 DNA reported in human tumors is very low, whereas SV40 is chromosomally integrated and present within each tumor cell in animals. Second, specific antibody responses against SV40 proteins are not found in NHL patients, even though systemic viral infections (including SV40 infection in animals) generally lead to antibody responses.
4. *Specificity.* This criterion specifies that a single cause lead to a unique effect. While this criterion is not absolute (e.g., smoking causes many different types of cancer and other diseases), the wide range of human tumors in which SV40 DNA reportedly has been detected is disconcerting.[14] Similarly, studies of NHL report detection of SV40 DNA in a wide range of NHL subtypes. Some laboratory studies of NHL and other tumors did not include negative controls or reported SV40 DNA in normal tissues, leading to further concerns regarding specificity.
5. *Consistency.* The evidence for a causal association is stronger if multiple studies of differing types all point to a relationship between exposure and outcome. The PCR-based laboratory studies reporting on the detection of SV40 in human NHL tumors are inconsistent (Table 1). In contrast, epidemiological evidence (cohort and case-control studies) consistently has been negative.
6. *Strength of association.* Association is measured using a relative risk or odds ratio. A strong association (i.e., large

TABLE 2
Hill criteria for simian virus 40 as a cause of non-Hodgkin lymphoma in humans

Hill criterion	Strength of evidence in favor of SV40 as a cause of cancer*	Comment
Biological plausibility	++	SV40 causes NHL in animals. SV40 has not been shown to infect human lymphocytes.
Analogy	++	SV40 causes NHL in animals.
Biological coherence	−	In animals, SV40 is chromosomally integrated in tumor cells, but DNA detection in human tumors is low-level. SV40-specific antibodies are not detected in NHL patients.
Specificity	−−	Wide range of tumors in which SV40 DNA has been reported.
Consistency	−−−	Laboratory studies reporting detection of SV40 in NHL tumors are inconsistent. Epidemiological studies are consistently negative.
Strength of association	−−−	Epidemiological studies consistently find a null association between SV40 exposure/infection and NHL.
Biological gradient	−	SV40-exposed infants are not at highest NHL risk.
Temporality	−−	Recipients of SV40-contaminated vaccines are not at increased NHL risk.
Experimental evidence	0	No data.

Criteria are from Hill.[39]

*Strength of evidence is presented on a scale from +++ (strong evidence in favor of a causal relationship) to −−− (strong evidence against a causal relationship).

relative risk or odds ratio) between SV40 exposure or infection and NHL would exist if NHL risk were much higher in SV40-exposed individuals than in SV40-unexposed individuals, or if NHL patients were much more likely to be SV40-infected than controls. Cohort studies of vaccine recipients and case-control studies of NHL utilizing serological markers for SV40 infection consistently have yielded null associations, providing no support for a causative relationship between SV40 and NHL. Studies reporting on the PCR-based detection of SV40 DNA in NHL tumors do not provide a measure of association.

7. *Biological gradient.* This criterion refers to a dose-response relationship. One way that this criterion can be assessed for NHL is to evaluate cancer risk in SV40-exposed infants, SV40-exposed older children, and SV40-unexposed individuals. SV40-exposed infants would be expected to have the highest risk and SV40-exposed children intermediate risk, based on data from animals showing highest risk with early age of infection.[24] Cohort studies of vaccine recipients have not identified an increased NHL risk in SV40-exposed neonates or infants.[20,22]
8. *Temporality.* For causality to explain an association, it is necessary that the exposure precede the outcome. With regards to SV40, the most important evidence regarding temporality comes from retrospective cohort studies of vaccine recipients. In those studies, the early-life exposure of these individuals to SV40 was not followed by an elevated incidence of NHL during extended follow-up.
9. *Experimental evidence.* By ''experimental evidence,'' Hill was referring not to laboratory data (e.g., experiments on cell cultures or animals) because these are relevant to the effects of SV40 in humans only indirectly, through plausibility or analogy. Rather, Hill was referring to experiments performed on humans.[39] There are no experimental data on humans that bear on the question of whether SV40 causes NHL.

Overall, consideration of these criteria suggests that the collected data supporting SV40 as a cause of NHL are not convincing. Several research approaches seem most likely to lead to new insights that could resolve lingering uncertainty regarding the role of SV40 in human cancer:[14]

1. *Improvements in laboratory methods for detection of SV40 in human tumors.* Improved laboratory methods are needed to address the specificity and reproducibility of reported detections of SV40 DNA in human tumors. Careful attention should be paid to the choice of PCR primers, ideally to target regions of the SV40 genome not included in plasmids.[15,18] Additionally, the use of quantitative PCR methods will help distinguish between the actual presence of virus and low-level PCR contamination. Of

importance, laboratory studies must include appropriate negative control tissues and incorporate identical treatment of tumor and control tissues, ideally using masking of specimens.[40]

2. *Wider use of serologic assays for SV40 infection.* Recently-developed enzyme immunoassays incorporating SV40 virus-like particles appear highly sensitive and specific and allow the identification of cross-reacting antibodies to BK or JC virus.[31] Refinements in these assays, development of other types of serologic assays, and their wider application will provide useful information regarding the epidemiology of SV40 and its possible associations with cancer.
3. *Additional studies of SV40 epidemiology in humans.* With improved molecular and serologic approaches, it will be possible to conduct studies to better determine the prevalence of SV40 infection in humans and identify potential routes of SV40 transmission.

Together, pursuit of these avenues of investigation may help resolve uncertainty regarding whether SV40 plays an etiologic role in NHL and other cancers. For instance, better standardization of molecular methods and direct comparison of PCR results with those of serology assays may provide a clearer understanding of the biological significance of the reported detections of SV40 DNA in human tumors. Similarly, studies of SV40 epidemiology in humans will help clarify whether SV40 DNA detected in tumors might reasonably be explained as deriving from SV40 infections. Given the widespread exposure to SV40 in poliovirus vaccines, the lingering question of whether SV40 causes cancer requires a definitive answer.

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